Research Note

Growth and Survival of Antibiotic-Resistant *Salmonella* Typhimurium DT104 in Liquid Egg Products

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ABSTRACT

Since 11 September 2001, quality and food safety are no longer the concerns of only consumers, industry, regulatory agencies, or other government officials. Liquid foods that are prepared or stored in bulk, including liquid egg products, are considered to be at potential risk for sabotage. Because of their versatility, low price, and functional properties, many of these products are being marketed. Four of the most common products of this type are whole egg, egg albumen, 10% sugared yolk, and 10% salted yolk. Although all of the serotypes of *Salmonella enterica* may cause illness, multidrug-resistant *Salmonella* Typhimurium DT104 has become widespread and can cause severe illness that is difficult to treat. Studies were conducted to determine growth patterns of *Salmonella* Typhimurium DT104 in four commercial liquid egg products held at 4, 10, 20, 30, 37, and 42°C for 0 to 384 h. All experiments were performed in duplicate and repeated twice. Standard methods were used to estimate cell numbers, and log CFU per gram of egg product was plotted against time. The number of cells of *Salmonella* Typhimurium DT104 increased to 8 to 9 log CFU/g in whole egg and 10% sugared yolk, increased by 1 log CFU/g in liquid albumen, but decreased by 3 log CFU/g in 10% salted yolk. Data from this study have been archived in the ComBase database to further assist policy makers or other scientists interested in *Salmonella* growth characteristics in liquid eggs. However, based on data generated in this study, *Salmonella* Typhimurium DT104 probably does not constitute a food threat agent in liquid eggs.

In the early 1900s, most egg production was a minor enterprise. However, automation has allowed producers to increase egg production (*3, 4*). In the late 1980s, developed countries around the world recorded eggborne outbreaks of salmonellosis due to *Salmonella enterica* serovar Enteritidis. Per capita egg consumption peaked in the 1940s at 405 and reached its lowest point (233.9) in 1991 (*1, 18*). Before passage of the 1970 Egg Products Inspection Act, several serotypes were associated with eggborne salmonellosis (*18*). *Salmonella* Typhimurium is commonly recovered from U.S. poultry and egg products (*8, 16, 17*). In recent years, this serotype and *Salmonella* Heidelberg have been increasingly recovered from egg-associated outbreaks of salmonellosis (*12*).

In 1984, an emerging *Salmonella* strain was first detected in the United Kingdom and has since been detected in many other developed countries around the world, including the United States. *Salmonella* Typhimurium DT104 is resistant to multiple antimicrobials and has been isolated from livestock and poultry in the United States. Of particular concern is the pattern of multiple antimicrobial resistance (R type) to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. The genes that encode for this resistance pattern are located on the chromosome, and removal of the selective pressure from antibiotics is unlikely to reverse resistance, as could occur with extrachromosomal-mediated resistance. In the United Kingdom, resistance to ciprofloxacin and trimethoprim also has been observed (*13*). Risk factors for *Salmonella* Typhimurium DT104 infection include consumption of undercooked meat and treatment with antibiotics (*7*). Worldwide increases in the incidence of multidrug-resistant *Salmonella* Typhimurium, including DT104, have been reported. Illness caused by these strains is more difficult to treat and results in greater morbidity and mortality (*11*).

Processed eggs accounted for 15% of egg production in 1984 but accounted for 31% of egg production in 2005, a 213% increase in just over 20 years (*2*). Further processing adds value to eggs, a very important development for commercial egg companies, which may generate a profit in only 2 or 3 years of a 5-year cycle (*4*). However, the popularity and versatility of these products and the nature of bulk processing and widespread distribution create the potential for food processing systems to be used as vehicles for the delivery of toxic or pathogenic agents in a terrorist attack (*21*).

As part of concerted efforts to prevent or prepare for an intentional attack on meat, poultry, and egg products, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has conducted vulnerability assessments of food systems. An approach designed by

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the U.S. Department of Defense, the CARVER + shock method, has been used by the USDA and the U.S. Food and Drug Administration to evaluate vulnerability in those food commodities. The FSIS has identified high priority areas for research and development pertaining to food defense, such as testing methods for certain threat agents. The FSIS also is working with the U.S. Department of Homeland Security National Biodefense Analysis and Countermeasures Center and the interagency Technical Support Working Group on several studies pertaining to the use of certain threat agents in food. Within the FSIS, the Office of Food Defense and Emergency Response manages all homeland security activities and has the responsibility of ensuring that all personnel, including administrators, policy makers, scientists, and field staff, are equipped to prevent and effectively respond to food security threats to egg, meat, or poultry production *(21)*.

As part of this effort, the growth, survival, and inactivation of antimicrobial-resistant *Salmonella* Typhimurium strains were studied over a broad range of storage temperatures. Data generated from these experiments will be used in the development of food security risk assessment programs and may be used in cases of unintentional recontamination of egg products, which are used as ingredients in other manufactured foods.

MATERIALS AND METHODS

Microbiological cultures. Studies were conducted to determine growth patterns of *Salmonella* Typhimurium DT104 in four commercial liquid egg products held at 4 to 42°C for 0 to 384 h. *Salmonella* Typhimurium DT104 strains 10 TX and 7470C-1, which were isolated from poultry and swine, respectively, were obtained from the Bacteriology, Epidemiology and Antimicrobial Resistance Research Unit (BEARRU) at the Richard B. Russell Research Center (Athens, GA). These isolates had been collected and characterized as part of the National Antimicrobial Resistance Monitoring System. Growth curves at a limited range of temperatures (20 to 42°C) were determined for *Salmonella* Typhimurium and *Salmonella* Heidelberg cultures isolated from commercial shell eggs *(16)*. These strains have demonstrated resistance to 11 to 13 antimicrobials *(17)*. An antimicrobial-resistant *Salmonella* Enteritidis isolate obtained from the BEARRU also was included in limited studies.

Egg products. Four commercial liquid egg products were obtained: pasteurized frozen whole eggs (FE; pH 7.6), pasteurized frozen high-whip eggs (FA: pH 9.1), pasteurized frozen sugared (10% sucrose) yolks (SuFY; pH 6.1), and pasteurized frozen salted (10% salt) yolks (SaFY; pH 6.0). No other ingredients were added to the products for processing of shelf life extension. These products were chosen because they are among the most commonly produced and consumed egg products in the United States *(2)*. Frozen egg products were obtained prepackaged in clean containers from commercial processing plants and transported frozen on ice to the laboratory. After products were allowed to thaw at 4°C, 50-g aliquots were placed in 120-ml sterile polypropylene specimen cups (Oxford, Mansfield, MA) and frozen at −20°C until analyses were performed. A single lot of each product was used for duplicate experiments.

Before inoculation, *Salmonella* Typhimurium DT104 stored at −80°C was grown at 37°C overnight on brilliant green sulfa (BGS) agar. This medium was supplemented with antibiotics to suppress any potential natural *Salmonella* present in the products. BGS agar was prepared according to the manufacturer's instructions (59 g/liter), and four antibiotics were added, each at 25 μg/ml: ampicillin, chloramphenicol, streptomycin, and tetracycline. Commercial liquid egg products are heat treated to achieve a 5-log reduction in *Salmonella* numbers, so it is unlikely that the organism would have been present before inoculation *(18)*. However, antimicrobial-resistant strains were used to eliminate the possibility that any naturally occurring *Salmonella* could confound the research data.

Experimental procedure. The day before each experiment, both *Salmonella* Typhimurium DT104 strains were plated from frozen stock onto BGS agar with antibiotics and incubated at 37°C. The four liquid egg products were removed from a −20°C freezer and allowed to thaw overnight at 4°C. Before inoculation, samples were kept at room temperature for approximately 1 h; after the inoculum was stirred into the samples, they were warmed to ~15°C.

On the day of the experiment, a swab was used to transfer cells from each *Salmonella* Typhimurium DT104 culture to a sterile tube containing phosphate-buffered saline (PBS), and the absorbance was measured to estimate bacterial concentration. A two-strain cocktail was prepared with equal amounts of each strain in a sterile PBS tube to reach the target inoculation level. Duplicate sterile sample cups were prepared by aseptically transferring 50 g of each egg product. Each sample cup was inoculated with 5×10^5 cells of the *Salmonella* Typhimurium DT104 cocktail (10^4 CFU/ml) and stirred with a sterile wooden stirrer for 30 s to ensure even distribution of inoculum.

After inoculation, sample cups were incubated at 4, 10, 20, 30, 37, or 42°C. Samples took 2 to 17 h to reach incubator temperatures. All experiments were repeated twice. Each incubator was fitted with a model FT121 temperature data recorder (Dickson, Addison, IL) attached to a thermocouple to monitor incubator temperature during the experiment. Samples were taken for plating at appropriate time intervals based on the expected growth rate for *Salmonella* at each experimental temperature (Figs. 1 through 6). An aliquot (0.1 g) of full-strength sample was spread plated on BGS agar with antibiotics, and then 1 g of liquid egg product was removed at each time interval and diluted 1:10 with sterile PBS for plating. Further serial dilutions were prepared with sterile PBS as necessary to facilitate enumeration. All plates were incubated at 37°C for 18 to 24 h before typical red colonies were counted, recorded, and log transformed to values for log CFU per gram of egg product. Viability data were modeled using DMFit curve-fitting software (Institute of Food Research, Norwich, UK) to determine the inactivation or growth rate and, when growth occurred, the lag period and maximum population density.

RESULTS AND DISCUSSION

Relatively little information has been collected concerning the feasibility of using microbial pathogens as threat agents in terrorist attacks on commercial food processing operations or in foods during processing or storage. An understanding of an organism's ability to survive and grow within food matrices is critical to focusing efforts to protect our food supply from malicious attack. Such information can be used in modeling programs that in conjunction with risk assessment can be used by homeland security groups for determining the feasibility of an attack, the most effective way to prevent an attack from occurring, or the best course of action in the event of an attack, ameliorating the deleterious effects to the health and economic well-being of industries and consumers *(13)*.
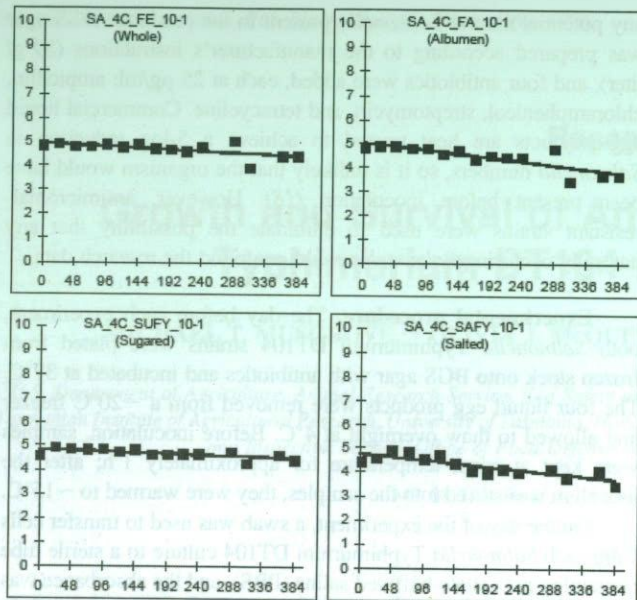
FIGURE 1. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 384 h at 4°C.*

FIGURE 3. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 120 h at 20°C.*

Eggs are highly nutritious for humans, providing lipids, minerals, vitamins, and high-quality proteins. These characteristics also make them excellent substrates for bacteria that can cause product spoilage or food poisoning *(18)*. As a result, a variety of treatments have been devised to decrease microbial counts in eggs and egg products *(6, 9, 10, 19)*. Facility sanitation, adherence to processing guidelines, and postprocessing cooling create hurdles that limit microbial contamination and growth *(14)*. Currently, the industry utilizes processing parameters that result in a 5-log reduction in *Salmonella (2)*. However, it is not clear how *Salmonella* intentionally added to products postpasteurization might persist or thrive at temperatures ranging from refrigeration to abuse.
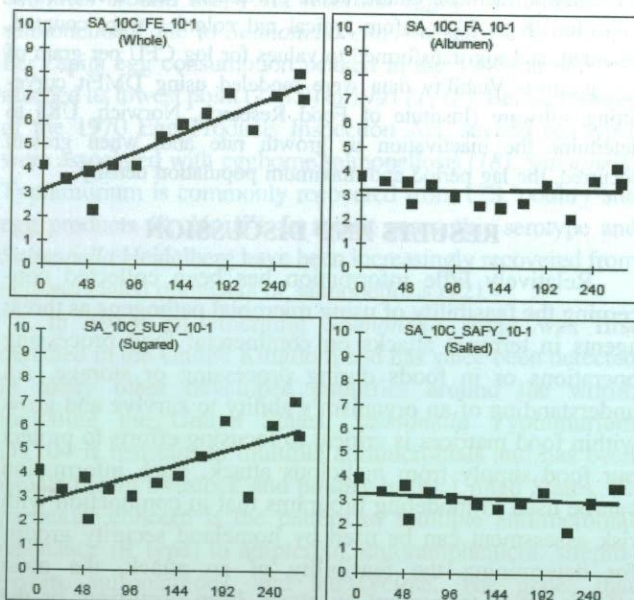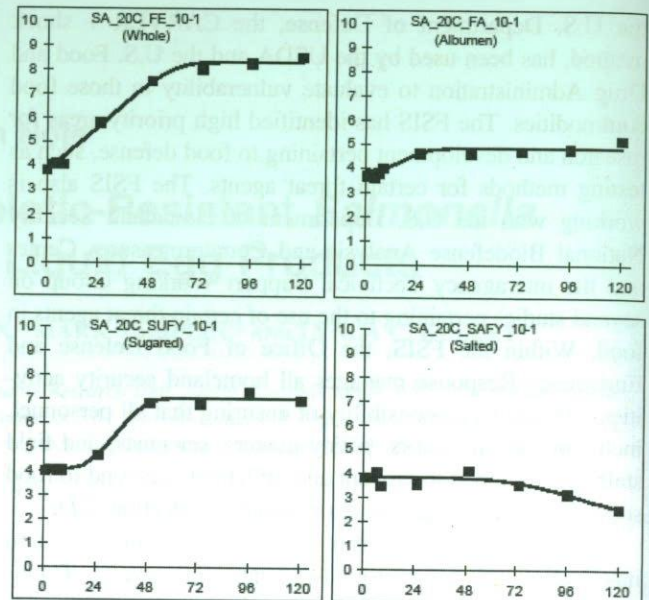
Results in Figures 1 through 6 represent data for whole egg, albumen, sugared yolk, and salted yolk held at 4, 10, 20, 30, 37, and 42°C. Growth was not observed in any of the products at 4°C, and *Salmonella* Typhimurium DT104 numbers decreased by 90% in albumen and 10% salted yolk during the 384 h of storage. At 10°C, growth was observed in whole egg and sugared yolk, but no change in viability was noted in the other two products during the 240 h. When eggs were held at 20°C for 120 h, there was a longer lag phase and a reduced maximum population density in sugared yolk compared with whole egg, and only 1 log CFU/g of growth was noted for *Salmonella* in albumen, with an approximate 99% reduction in salted yolk. A similar
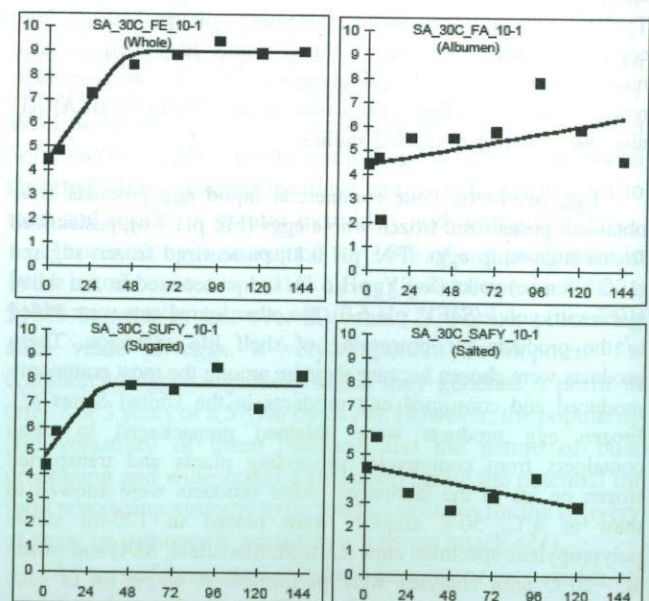

FIGURE 2. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 240 h at 10°C.*

FIGURE 4. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 144 h at 30°C.*
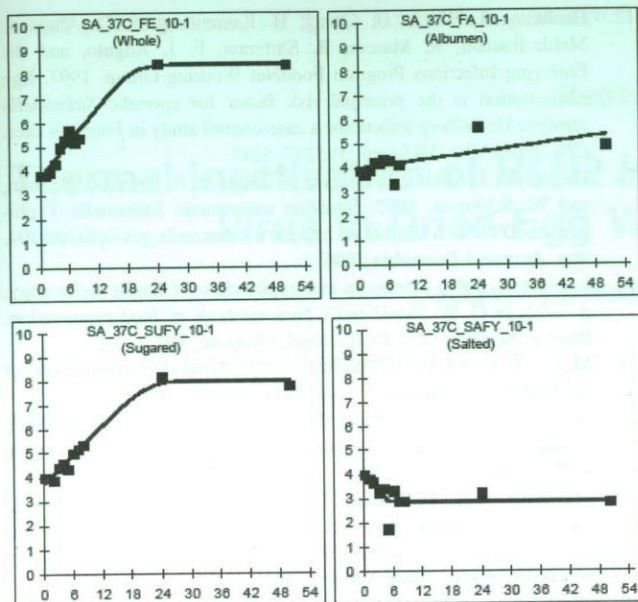
FIGURE 5. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 54 h at 37°C.*

trend in *Salmonella* behavior in the four products was observed at 30, 37, and 42°C.

Additional *Salmonella* serotypes (Typhimurium, Enteritidis, and Heidelberg) were studied at 20°C and displayed growth patterns very similar to those for *Salmonella* Typhimurium DT104 (data not shown). When the products were held at 42°C, *Salmonella* Typhimurium DT104 reached 9 log CFU/g in whole egg but displayed a longer lag phase than that in sugared yolk. There was no appreciable growth in albumen and a 99.9% reduction in numbers in salted yolk during the 30-h storage period.

Garibaldi et al. *(9)* reported that different components in egg products confer varying levels of heat resistance to
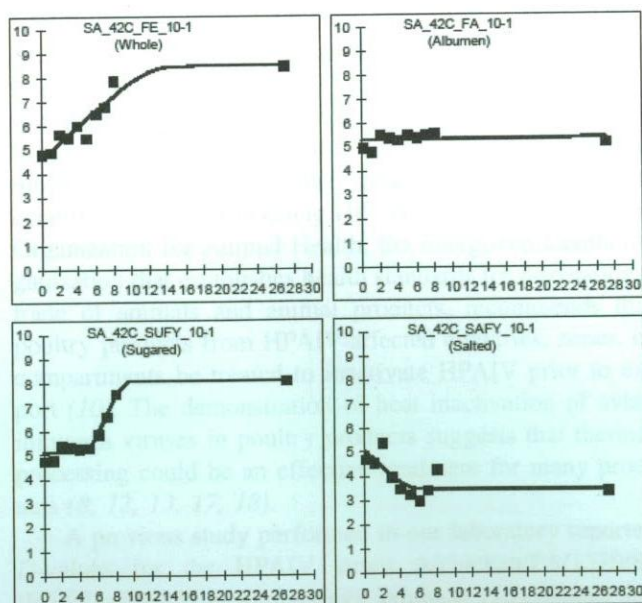

FIGURE 6. *Growth of* Salmonella *Typhimurium DT104 (log CFU per gram) in whole egg, albumen, 10% sugared yolk, and 10% salted yolk incubated for 0 to 30 h at 42°C.*

bacteria. Additional carbohydrate (10% sucrose) in the yolk increased the ability of the *Salmonella* to survive 60°C pasteurization. However, in the current study, sucrose did not appear to have an effect on *Salmonella* growth and survival during storage.

Little *Salmonella* growth occurred in albumen. There are many factors that limit the ability of microorganisms to survive and grow in this material *(5, 15)*. The viscous nature of albumen proteins, particularly when eggs are freshly laid, makes microbial motility difficult. This viscosity coupled with a lack of available water and nutrients make albumen inhospitable to bacterial growth *(22)*. However, the main impediment to bacterial growth or survival in albumen is chemical. Several important antimicrobial compounds naturally occur within albumen. Compounds such as ovotransferrin and conalbumin chelate metal ions, particularly iron, ovomucoid inhibits trypsin, and lysozyme causes hydrolysis of β-1,4-glycosidic bonds in peptidoglycans. Ovoinhibitor inactivates several proteases, ovoflavoprotein chelates riboflavin, and avidin binds biotin.

Albumen pH is alkaline. Immediately after oviposition, the pH of the albumen ranges from 7.6 to 7.9, but a gradual increase occurs during storage. As carbon dioxide diffuses out of the egg, the pH increases to greater than 9, beyond the growth limit of most microorganisms. Lysozyme, conalbumin, and pH are considered the most important antimicrobial factors naturally occurring in albumen *(15)*.

Addition of salt to foods has been used as a means of preservation for centuries *(20)*. Water activity is decreased by the addition of salt, making microorganisms more susceptible to other stresses such as temperature or the presence of competing organisms. The use of multiple means of microbial suppression in a food or process is referred to as hurdle technology. The present study results indicate clearly how effective salt can be at minimizing bacterial contamination of food.

Although *Salmonella* numbers increased to 8 to 9 log CFU/g in whole egg and sugared yolk, viable numbers increased only slightly in albumen and were decreased by as much as 3 log CFU/g in salted yolk at 42°C. Refrigerated tanker trucks are used to transport liquid egg products. Transportation is probably the most vulnerable food chain link for intentional contamination because a tanker truck may hold more than 20,000 gallons (75,680 liters) of liquid egg products, allowing a large number of people to be potentially exposed or harmed. However, the volume of inoculum required for a tanker truck would be so large that the feasibility of such an attack is questionable, and recommended cooking temperatures for eggs (71.1°C or above) would destroy *Salmonella* Typhimurium DT104 *(2)*.

Data generated with these experiments have been deposited in ComBase to assist with the development of predictive models and risk assessment.

ACKNOWLEDGMENTS

REFERENCES

1. American Egg Board. 2007. Egg industry fact sheet. Available at: http://www.aeb.org/Industry/Facts/FactsSheet.htm. Accessed March 2007.

2. American Egg Board. 2007. Egg solutions: the complete reference for egg products. Available at: http://www.aeb.org/EggProducts/. Accessed March 2007.

3. Bell, D. D. 1995. Forces that have helped shape the U.S. egg industry: the last 100 years. Poult. Trib. 1:30–43.

4. Bell, D. D. 2002. Introduction to the U.S. table-egg industry, p. 945–964. In D. D. Bell and W. D. Weaver, Jr. (ed.), Commercial chicken meat and egg production, 5th ed. Kluwer Academic, Norwall, MA.

5. Board, R. G., and H. S. Tranter. 1995. The microbiology of eggs, p. 81–103. In W. J. Stadelman and O. J. Cotterill (ed.), Egg science and technology, 4th ed. Food Products Press, New York.

6. Brackett, R. E., J. D. Schuman, H. R. Ball, and A. J. Scouten. 2001. Thermal inactivation kinetics of Salmonella spp. within intact eggs heated using humidity-controlled air. J. Food Prot. 64:934–938.

7. Doorduyn, Y., W. E. VandenBrandhop, Y. T. H. P. Vanduynhoven, W. J. B. Wannet, and W. Vanpelt. 2006. Risk factors for Salmonella Enteritidis and Typhimurium (DT104 and non-DT104) infections in the Netherlands: predominant roles for raw eggs in Enteritidis and sandboxes in Typhimurium infections. Epidemiol. Infect. 134:617–626.

8. Dreesen, D. W., H. M. Barnhart, J. L. Burke, T. Chen, and D. C. Johnson. 1992. Frequency of Salmonella enteritidis and other salmonellae in the ceca of spent hens at the time of slaughter. Avian Dis. 36:247–250.

9. Garibaldi, J. A., R. P. Straka, and K. Ijichi. 1969. Heat resistance of Salmonella in various egg products. Appl. Microbiol. 17:491–496.

10. Glass, K., M. Ceizik, K. Kaufman, A. Duerr, A. Irawan, and E. Johnson. 1997. Microbiological safety of egg products. Food Research Institute, Madison, WI.

11. Helms, M., S. Ethelberg, K. Molbak, and the DT104 Study Group. 2005. International Salmonella Typhimurium DT04 infections, 1992–2001. Emerg. Infect. Dis. 11:859–867.

12. Hennessy, T. W., L. H. Cheng, H. Kassenborg, S. D. Ahuja, J. Mohle-Boetani, R. Marcus, B. Shiferaw, F. J. Angulo, and the Emerging Infections Program FoodNet Working Group. 1997. Egg consumption is the principal risk factor for sporadic Salmonella serotype Heidelberg infections: a case-control study in FoodNet sites. Clin. Infect. Dis. 38(Suppl. 3):5237–5243.

13. Hogue, A., J. Akkina, F. Angulo, R. Johnson, K. Peterson, P. Saini, and W. Schlosser. 1997. Situation assessment: Salmonella Typhimurium DT104. Available at: http://www.fsis.usda.gov/ophs/stdt104.htm. Accessed December 2006.

14. Leistner, L. 1994. Principles and applications of hurdle technology, p. 1–21. In G. W. Gould (ed.), New methods of food preservation. Blackie Academic and Professional, Glasgow, UK.

15. Mayes, F. J., and M. A. Takeballi. 1983. Microbial contamination of the hen's egg: a review. J. Food Prot. 46:1092–1098.

16. Musgrove, M. T., D. R. Jones, J. K. Northcutt, and N. A. Cox. 2005. Impact of commercial processing on the microbiology of shell eggs. J. Food Prot. 6:2367–2375.

17. Musgrove, M. T., D. R. Jones, J. K. Northcutt, M. A. Harrison, N. A. Cox, P. J. Fedorka-Cray, and S. R. Ladely. 2006. Antimicrobial resistance patterns of Salmonella isolated from commercial shell egg processing plants. Poult. Sci. 85:3001–3004.

18. Ricke, S. C., S. G. Birkhold, and R. K. Gast. 2001. Eggs and egg products, p. 473–479. In F. P. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.

19. Swain D. E., and J. R. Beck. 2004. Heat inactivation of avian influenza and Newcastle disease viruses in egg products. Avian Pathol. 33:512–518.

20. Toussaint-Samat, M. 1992. History of food. Blackwell Publications, Cambridge.

21. U.S. Department of Agriculture, Food Safety and Inspection Service. 2007. Food defense and emergency response. Available at: http://www.fsis.usda.gov/Food_Defense_&_Emergency_Response/Preparation_&_Prevention/index.asp. Accessed March 2007.

22. Yadav, N. K., and D. V. Vadehra. 1977. Mechanism of egg white resistance to bacterial growth. J. Food Sci. 42:97–99.